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Change in the surface density of immobilized enzyme molecules due to photoelectron processes in a silicon substrate

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Atomic force microscopy (AFM) and Kelvin probe force microscopy (KPFM) are an effective methods for monitoring the surface of hybrid and biosensor structures based on silicon substrate and organic components (such as enzyme, DNA, bacteria, etc.).

In our work, AFM and KPFM has been used to study of influence of both the Si illumination during enzyme deposition and salt concentration in enzyme solution on the density of adsorbed enzyme molecules.

The experiments were performed with single-crystal silicon wafers of n-type ($\rho \cong 4\ \Omega$ cm) and p-type ($\rho \cong 8\ \Omega$ cm) with layer of amorphous silicon (a-Si). Initially, the substrates were boiled in a peroxide–ammonia solution and rinsed in deionized water (resistivity 18 MΩ). This treatment leads to "reconstruction" of a native oxide layer while the silicon surface acquires negative charge in deionized water due to activation of OH-groups. Glucose oxidase (GOx) molecules from *Aspergillus niger* was used as enzyme molecules. In a wide pH range of the solution, the GOx molecule have an effective negative charge. The size of the GOx molecule is $6.0\times5.2\times7.7$ nm^3 [1]. A cationic polyelectrolyte polyethylenimine (PEI) with a molecular weight of 25 kDa was used to increase the adsorption of negatively charged GOx onto silicon substrates. The PEI molecules were adsorbed onto silicon substrates from the 1 mg/ml aqueous solution during 10 min followed by rinsing in water during 10 min and drying. The photo-assisted layer-by-layer adsorption technique suggested in [2] was used to adsorb GOx from the 0.5 mg/ml aqueous solution of both with and without NaCl onto bare substrates and covered with PEI silicon substrates. The NaCl concentration was varied from 0.01 M to 0.1 M.

The topography and surface potential of the films were measured using AFM and KPFM by NTGRA Spectra (NT-MDT Spectrum Instruments, Russia). Scanning was performed under ambient conditions at a frequency of 0.5 Hz in taping mode using HA_NC/W_2C cantilevers of ETALON series. The Gwyddion software for statistical analysis of AFM data was used.

Analyzing AFM micrographs of adsorbed GOx molecules on p-Si/a-Si and n-Si/a-Si substrates, we found that adding a small amount of salt to the GOX solution results in a decrease in adsorption. However, further an increase in the salt concentration in the solution leads to an increase in both the number of adsorbed enzyme molecules and the size of the irregularities. Without illumination, the amount of adsorbed particles is larger on the p-Si/a-Si/PEI structure and smaller on the n-Si/a-Si/PEI structure. However, the illumination of the silicon substrate during the adsorption process alters the adsorption significantly: on the p-Si/a-Si/PEI surface, the number of adsorbed particles decreases, while on the p-Si/a-Si/PEI surface, there is a pronounced increase in the amount of adsorbed particles. These data correlate with the KPFM data.

Thus, using the methods of scanning probe microscopy, it has been shown that the adsorption of enzyme molecules is substantially depends on the ionic strength of the solution, the conductivity type of Si substrate as well as on photoelectron processes in semiconductor.

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